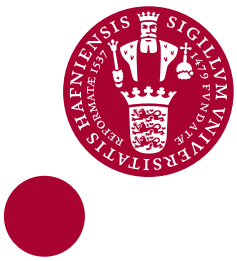

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Robustness of a cross contamination model describing transfer of pathogens during grinding of meat

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Abstract

This study aimed to evaluate a cross contamination model[1] for its capability of describing transfer of *Salmonella* spp. and *L. monocytogenes* during grinding of varying sizes and numbers of pieces of meats in two grinder systems. Data from 19 trials were collected. Three evaluation approaches were applied: *i*) Acceptable Simulation Zone method compared observed with simulated transfer, *ii*) each trial was fitted and parameters were integrated in a Quantitative Microbiological Risk Assessment model, *iii*) the Total Transfer Potential was calculated from fitted parameters. Risk estimates revealed that grinding was influenced by sharpness of grinder knife, specific grinder and grinding temperature.

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Keywords: microbial transfer; foodborne pathogens; model assessment; risk; meat processing.

1. Introduction

Møller et al. (2012)[1] published a model capable of describing the observed transfer of *S.* Typhimurium DT104 during grinding of pork. It is not known whether the model is equally capable of describing transfer of different pathogens in other meat matrices using different grinding systems. Therefore, the aim of this study was to evaluate

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the capability of this model to properly describe the transfer of both *Salmonella* spp. and *L. monocytogenes* when grinding different types of meat (pork and beef), using two different types of grinders and variable sizes and numbers of meat pieces to be minced.

2. Material and methods

2.1. Experimental design

As indicated in Table 1, microbial transfer was investigated in relation to types of meat (beef and pork), piece sizes (50 to 324 g) and number of pieces subjected to grinding (10 to 100), as well as three bacterial pathogens (*S.* Enteritidis, *S.* Typhimurium DT104 and *L. monocytogenes*).

Table 1. Aspects challenged in each of the performed experiments and in the published datasets

Trial	Meat Type	Inoculation		Pieces of meat		Temperature of processing (°C)
		Pathogens	Concentration (log_{10} CFU/piece)	Size (g)	Number	
1	beef[a]	*S.* Enteritidis cocktail[c]	6.85[d]	50	90	19 - 27
2	beef[a]	*S.* Enteritidis cocktail	6.86[d]	50	90	19 - 27
3	beef[a]	*S.* Enteritidis cocktail	7.48[d]	50	80	19 - 27
4	beef[a]	*S.* Enteritidis cocktail	7.74 - 8.26	50	80	19 - 27
5	beef[a]	*S.* Enteritidis cocktail	7.92 - 8.00	50	80	19 - 27
6	beef[a]	*S.* Enteritidis cocktail	7.74 - 8.30	50	80	19 - 27
7	beef[a]	*S.* Enteritidis cocktail	6.80 - 7.65	50	24	19 - 27
8	beef[a]	*S.* Enteritidis cocktail	6.78 - 8.04	50	24	19 - 27
9	pork[a]	*S.* Enteritidis 54	6.33 - 8.48	196 ± 35	100	22 - 27
10	pork[a]	*S.* Enteritidis 54	8.11 - 8.77	196 ± 25	10	22 - 27
11	pork[a]	*S.* Enteritidis 54	8.07 - 8.82	186 ± 29	96	22 - 27
12	pork[a]	*S.* Enteritidis 54	7.70 - 8.50	157 ± 26	15	22 - 27
13	pork[b]	*S.* Typhimurium DT104	8.32 - 9.00	170 ± 46	25	22
14	pork[b]	*S.* Typhimurium DT104	8.71 - 8.92	229 ± 63	25	22
15	pork[b]	*S.* Typhimurium DT104	8.33 - 9.10	224 ± 61	35	22
16	pork[b]	*S.* Typhimurium DT104	8.61[e]	274 ± 37	44	22
17	pork[b]	*L. monocytogenes*	8.76[e]	324 ± 53	45	22
18[f]	pork[b]	*S.* Typhimurium DT104	9.10 - 9.52	236 ± 64	45	22
19[f]	pork[b]	*S.* Typhimurium DT104	8.87 - 9.33	241 ± 49	45	4
20[f]	pork[b]	*S.* Typhimurium DT104	8.71 - 9.22	230 ± 49	95	4

[a] processing in a semi-industrial grinder in stainless steel and tin (Beccaro® equipamentos industriais Ltda, Brazil (Model Picador PB-10I)
[b] processing in a semi-industrial grinder in stainless steel (la Minerva® food service equipment, Italy (Model AE22)
[c] a strain of *S.* Enteritidis isolated from beef and another *S.* Enteritidis strain isolated from chicken legs were tested in this cocktail.
[d] for modelling purposes, the input of the pathogen was estimated based on counts directly from the culture.
[e] for modelling purposes, the average of the input of the pathogen to all five contaminated pieces of meat was applied.
[f] data obtained from Møller et al. (2012)[1].

Following the methods of Møller et al. (2012)[1], five pieces of experimentally contaminated pieces were ground, followed by non-contaminated pieces. Individual portions of each ground piece were collected and analyzed.

2.2. Model

Parameter values of the he cross contamination model proposed and explained by Møller et al. (2012)[1] (equation.1) were estimated by fitting the observed values from each of the twenty trials (Table 1) by minimizing the Residual Sum of Squares (RSS), using the Solver function in MS Excel (Microsoft® Office Excel® 2007).

$$\begin{cases} M_i = (1-a_1)(1-a_2)\,P_i + (b_1\,gr_{1,i-1}) + (b_2\,gr_{2,i-1}) \\ gr_{1,i} = a_1\,P_i + (1-b_1)\,gr_{1,I-1} \\ gr_{2,i} = a_2\,P_i + (1-b_2)\,(1-c_3)\,gr_{2,i-1} \end{cases} \quad \text{(Equation 1)}$$

2.3. Evaluating model performance

In order to evaluate the robustness of the Møller et al. (2012)[1] transfer model, three approaches were applied:

- Assessment through Acceptable Simulation Zone (ASZ) concept:

The parameter estimates obtained in trial 20 (Table 2) were used in Eq. 1 to simulate the cross contamination events for the remaining 19 trials shown in Table 1. The observed values for the simulations of the cross contamination events of the 19 trials were then compared to the simulated values by applying the ASZ concept[2,3].

- Assessment through a Quantitative Microbiological Risk Assessment (QMRA) approach:

A QMRA model[4] was used to evaluate the impact of the cross contamination events during grinding in each of the 20 trials on the risk estimates of salmonellosis due to consumption of Danish meatballs produced from ground meat. The entire QMRA model was simulated with the Monte Carlo technique (10,000 iterations) using @Risk (version 5.7, Palisade, Newfield, NY, U.S.). The simulation was repeated five times for each tested set of parameter estimates for cross contamination, and the results expressed as the mean risk per serving of one meatball.

- Assessment through the Total Transfer Potential:

The total transfer potential (TTP) is defined as the proportion of bacterial cells that is transferred from a single slice of meat (S_1) to the ground meat ($\sum M_{i=1}^{\infty}$). Rewriting equation (1) shows that

$$TTP = \frac{\sum_{i=1}^{\infty} M_i}{S_1} = \left(1 - a_2\left(1 - a_1 - \frac{b_2}{c_3 + b_2(1 - c_3)}\right)\right) \times 100\% \qquad \text{(Equation 2)}$$

TTP was calculated for each of the 20 fitted datasets (Table 2). It indicates the percentage (%) of CFU of pathogen, from the contaminated meat that ends up in the total minced meat, assuming that the grinding process will continue forever. In practice, the grinding will not be performed forever and, therefore, the % obtained with Eq. 2 will be a little overestimated, which is not relevant since the main bacterial loads in this study are added at the beginning of the processing. It is expected that a higher % of TTP implies a higher risk.

3. Results and Discussion

3.1. Correlation between approaches applied for model evaluation

It is difficult to evaluate the performance of a model for a non-linear process, described by a number of parameters[5]. We propose a method for model evaluation by applying and correlating three assessment approaches.

The aspects considered for this evaluation were: 1) the width of the Acceptable Simulation Zone (ASZ) necessary to include 95 % of the predicted values in the transfer curve obtained with parameters from trial 20; 2) the risk estimates of salmonellosis by consumption of catered Danish meatballs, obtained applying the QMRA approach; and 3) the Total Transfer Potential percentage (TTP%) suggested in this study (Eq. 2).

It was found that in general there was a good correlation between the approaches applied in this study, especially between TTP% and the risk estimates (Table 2). Hence, in general, TTP% may serve as a nice tool to quickly obtain a risk estimate without running a complex QMRA model.

Comparison between trials indicates that extreme values were found to be ASZ higher than 1.1 $\log_{10}$CFU per portion, Risk Estimates higher than 2.1 x 10^{-3}, and TTP% similar to or higher than 60 %. These Extreme values were obtained applying all evaluation approaches by trials 1 – 3, (performed with dull knife), 12 (15 portions averaging 157 g) and 14 (25 portions). Two of the applied approaches had extreme values for trial 9 (performed with dull knife). At least one of the applied approaches had extreme values for trials 5, 6, 8 (portions averaging 50 g), 10 (10 portions), 13 (25 portions averaging 170 g), 16 (portions averaging 274 g), and 17 (portions averaging 324 g).

Table 2. Summary of performance results (with extreme values in **bold**) obtained with three different approaches for evaluating performance of the model proposed by Møller et al. (2012)[1] describing the transfer of pathogens during meat grinding.

Trial	Parameter estimates from fitting					RMSE[b]	Size of ASZ[c] to include 100 % of the predictions (± CFU/portion of meat)	Absolute Risk[e] Estimates x 10^{-3}	TTP %[f]
	a_1	b_1	a_2	b_2	1-c_3				
1	0.0558	0.0544	0.4946	0.4175	1.0000	1.2038	**2.0**	**3.02**	**103**
2	0.0503	0.0454	0.1707	0.3522	1.0000	1.1536	**2.0**	**2.87**	**101**
3	0.0335	0.0569	0.0612	0.1485	1.0000	1.0754	**2.0**	**2.94**	**100**
4	0.0041	0.0471	0.8778	0.0570	0.4641	1.1018	0.8	1.01	21
5	0.0118	0.0652	0.7787	0.0927	0.5058	1.0760	**1.4**	1.56	36
6	0.0104	0.0677	0.8330	0.0588	0.3004	1.1079	**1.2**	1.10	24
7	0.0092	0.1472	0.8697	0.0261	0.1459	1.1879	1.1	0.86	16
8	0.0107	0.0265	0.9388	0.0253	0.4024	1.1508	**1.2**	0.62	11
9	0.0167	0.0061	0.5386	0.0579	0.8083	1.2202	**2.0**	**2.21**	60
10	0.4227	0.0052	0.4250	0.2715	0.5905	2.0930	0.7	1.94	**96**
11	0.0047	0.0358	0.6549	0.1251	0.5479	1.1744	1.1	1.92	51
12	0.0799	0.3165	0.2611	1.0000	0.0000	1.4371	**1.4**	**3.04**	**102**
13	0.0293	0.2433	0.8785	0.1648	0.3284	1.1196	**1.2**	1.44	35
14	0.0054	0.1127	0.5721	0.1653	0.6541	1.1802	**1.5**	**2.22**	**64**
15	0.0054	0.1584	0.7369	0.0484	0.6276	1.1909	1.1	1.54	36
16	0.0130	0.1134	0.5289	0.1304	0.4963	1.1451	1.1	**2.24**	60
17	0.0142	0.1022	0.7746	0.1767	0.1588	1.2773	**2.2**	1.59	39
18[a]	0.0008	0.0655	0.7924	0.1331	0.2475	1.2029	0.6	1.41	34
19[a]	0.0020	0.0809	0.8166	0.0555	0.3692	1.1345	0.6	1.14	25
20[a]	0.0010	0.0275	0.8909	0.0558	0.4887	1.1378	NA[d]	0.96	20

[a] data published by Møller et al. (2012).
[b] Root Mean Sum of squared Errors.
[c] ASZ – Acceptable Simulation Zone, proposed by Oscar in 2005[2] and tested by Møller et al. in 2013[3].
[d] NA – Not Applicable, because the parameters applied to access the ASZ were obtained by the fitting of trial 20.
[e] risk estimates from scenarios testing different sets of transfer parameters (Table 2), and using the QMRA of *Salmonella* in meatball processing model (Møller et al., 2015[4]) at low concentration and prevalence of the pathogen.
[f] Calculated with the equation derived from Møller et al. (2012)[1]. It indicates the percentage (%) of CFU of *Salmonella*, from the contaminated grinded pieces that ends up in the total minced meat, assuming that the grinding process will continue forever.

4. Conclusions

By applying the suggested method for model evaluation, considering an agreement of the results obtained in at least two of the three assessment approaches, it was found that parameter estimates obtained by fitting one grinding process may not be applied to describe transfer of pathogens during grinding under different conditions. Nevertheless, the risk estimates revealed that the risk of foodborne disease was reduced when the grinding of meat was performed in a grinder made of stainless steel (in all surfaces of the machine in contact with the meat), using a well-sharpened grinder knife, and holding at room temperatures of 4°C or lower.

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